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Antioxidant Capacity, Physicochemical and Floral Characterization of Honeys from the Northeast of Brazil

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Capacidade Antioxidante, Caracterização Físico-química e Floral de Méis do Nordeste do Brasil

Resumo: O objetivo deste trabalho foi avaliar a origem floral, a qualidade físico-química e a capacidade antioxidante de 15 amostras de mel, da região semi-árida do nordeste do Brasil e correlacionar os dados com a cor e o conteúdo total de fenóis (TPC). Observou-se uma predominância da cor âmbar. A maioria das amostras foi classificada como polifloral e, apenas duas amostras (amarelo claro) foram de origem monofloral. Os valores de TPC variaram de 27,0 a 92,7 mg EAG 100 g^{-1} de mel, sendo mais elevados em amostras mais escuras. Todas as amostras mostraram capacidade de sequestrar radicais $DPPH^{\bullet}$ e poder redutor analisado pelos métodos de FRAP e CUPRAC, com melhor desempenho em amostras mais escuras. O TPC apresenta boa correlação ($p<0,05$) com os resultados relacionados à capacidade antioxidante. Foram observadas correlações significativas ($p<0,01$) entre a cor, o TPC (0,82) e também com o poder redutor, via CUPRAC (0,66). A cor mais escura do mel é um indicativo de maior teor de fenóis e maior capacidade antioxidante. As características aqui descritas enriquecem as propriedades de alimento funcional do mel e contribuem para o desenvolvimento da economia regional, fornecendo alternativas para a geração de renda familiar regional.

Palavras-chave: Teor total de fenóis; capacidade antioxidante; análise físico-química; mel.

Abstract

The aim of this work was to evaluate floral origin, physicochemical quality and antioxidant capacity of 15 honey samples, from a semi-arid region in the northeast of Brazil and to correlate data with color and total phenolic content (TPC). A predominance of amber color was observed. The majority of samples were classified as polifloral and only two samples (the light yellow ones) were monofloral. TPC values ranged from 27.0 to 92.7 mg GAE 100 g^{-1} of honey, being higher in darker samples. All samples had ability to scavenge $DPPH^{\bullet}$ radicals and showed reducing potential analyzed by FRAP and CUPRAC methods, with the highest performance obtained in darker samples. TPC shows good positive correlations ($p<0.05$) with the methods used to evaluate antioxidant capacity. Significant correlations ($p<0.01$) between color and TPC (0.82) and also with reducing power, using CUPRAC (0.66) were observed. The darker color of honey is indicative of higher phenol content and antioxidant capacity. The reported characteristics give an enhanced functional food property to honey and contribute to the development of the regional economy, providing an alternative to family income increase.

Keywords: Total phenolic content; antioxidant capacity; physicochemical analysis; honey.

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Antioxidant Capacity, Physicochemical and Floral Characterization of Honeys from the Northeast of Brazil

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1. Introduction

Honey is the organic, natural sugar, produced from the nectar and exudation of plant by honey bees.[1] It is mainly composed by sugars (fructose and glucose), water and also contains small amounts of other constituents like proteins, vitamins, minerals, flavonoids, phenolic acids, enzymes, numerous volatile compounds and other natural products.[2-6] Among the chemical constituents, it contains a variety of organic compounds with antioxidant and radical-scavenging ability[7] and contributes to human health and nutrition, being considered a functional food.[3,7-12]

Othman[13] recently referred to an inverse honey and cancer relationship, showing sizeable evidences that honey is a natural immune booster, anti-inflammatory agent, antimicrobial agent, cancer "vaccine," and promoter for healing chronic ulcers and wounds. It was also reported various possible mechanisms by which honey may inhibit growth and proliferation of tumors or cancers.[14] Recent data suggest that honey, administered alone or in combination with conventional therapy, might be a novel natural antioxidant in the management of chronic diseases commonly associated with oxidative stress.[15] On the other hand, honey may have undesirable effects on health.[8] It can be expected to contain a small number and a limited variety of microorganisms and several heavy metals such as Sb, As, Cd(II), and Pb(II)[8] and contaminants, like pesticides and others. Due to that, some precaution must be taken for their use in foods to, also, avoid some problems in persons who suffer from allergy by bee related allergens.[8]

The quality of honey depends upon its physicochemical and sensory properties.[16] The composition of the different types of honey varies with different floral sources, bee species, as well as climatic and environmental conditions, along with the type of product processing and quality of beekeeper.[2,17-19] Even if it is made from the same floral origin or the same bee species, honey can vary in texture, color, and composition depending on the geographical origin, soil, weather conditions, and even the age of the bees, which greatly affects the enzymatic activity that produces the honey.[20] In order to determine the legitimacy of the particular floral source of honey, analysis of pollen (melissopalynology) and either organoleptic or physico-chemical properties are traditionally employed.[21] Their mineral, trace elements and ash content and electrical conductivity data were repeatedly shown to be a very effective means for the classification purposes of honey of various origins (geographical and botanical).[21] Reliable techniques for origin authentication are essential because official analysis of honey involves a series of several determinations of chemical and physical parameters that will be of little use for the geographical certification. Therefore, in recent years, several efforts have been made to address authenticity, typicality, traceability and intrinsic quality of honeys with the application of multivariate statistical techniques.[21,22] Additionally, fluorescence spectroscopy coupled with parallel factor analysis (PARAFAC) and partial least squares discriminant analysis (PLS DA),[23] along with GC/MS fingerprinting of headspace volatile compounds, subjected to orthogonal partial least squares-discriminant analysis™ (OPLS™-DA), soft independent modelling of class analogy (SIMCA), and OPLS™-hierarchical cluster analysis (OPLS™-HCA),[24] were used for characterization and classification of honey. Low Field (LF) 1H Nuclear Magnetic Resonance (1H NMR), a rapid method for general application, to investigate water mobility in materials and foods, was used, as well, for differentiating the botanical origin of honeys.[25] Analytical possibilities of NMR measurements for compositional analysis as well as NMR metabonomics and component profiling for determining the authenticity of honey, along with use of Near Infrared, FT-Raman have enriched the arsenal of analytical chemist in this direction. [26,27] Several other methods are available, however, the application of these techniques has to be still developed and systematization

is necessary and urgent as an important part of authenticity assessments.

The therapeutic value (antibacterial and anti-inflammatory properties) of honey has been partially attributed to its antioxidant properties.[3,19,28-31] Its nutraceutical and antimicrobial properties against multidrug-resistant strains can be also related to the presence of polyphenols.[32,33] It was reported that the antioxidant capacity depends on the floral source and on seasonal and environmental factors,[34-36] as well as on the method of processing honey.[37] Several methods have been applied, in recent years, to evaluate the antioxidant capacity of honey samples.[38,39]

In Brazil, the most common bee is the Africanized bee, which is a hybrid between the African and European *Apis mellifera* honeybee species.[20] The cashew honey from Piauí was shown to be dark colored and to have pronounced acidity and a relatively high amount of amino acids, so, the geographical origin of honey is an important parameter in this area of research.[40] In the semi-arid region of northeast of Brazil, there is a diversified flora. In the Bahia State, many types of farmhouse honey are produced. Bahia honey varieties span a wide range of originating flora and microclimatic and atmospheric conditions. This substantially increases their market value and raises the need to detect potential frauds in origin or even adulteration.[21]

In spite of this, the physicochemical, chemical, and biological properties of honey are still poorly explored. In micro-region of the Ribeira do Pombal (Bahia state from Brazil) composed of 17 municipalities, produced in 2010, 826.74 tons of honey[41] which correspond to 34.5% of total production in this state. More than 80 plants were identified as a bee flora, being the Fabaceae family predominant.[42] As said, honey has antioxidant capacity, however, up to our knowledge, few reports of the antioxidant characteristics of Brazilian honeys, especially from the Northeast of Brazil has been so far published. Thus, the aim of this work was to evaluate floral origin, physicochemical quality and antioxidant capacity of 15 honey samples and to correlate resulting data with color and the total phenolic content of the samples. The importance of honey has been continuously upgrading because of its nutrient and therapeutic effect, which, in turn is directly related on its phenolic content and antioxidant capacity.

2. Materials and Methods

2.1. Standards and reagents

Folin-Ciocalteau reagent, ethanol, methanol, DPPH$^{\bullet}$ (α,α-diphenyl-β-picrylhydrazyl radical) and copper (II) chloride, were purchased from Sigma Aldrich (Steinheim, Germany). Sodium carbonate and gallic acid (GA) were supplied by Vetec Química Fina Ltda (Rio de Janeiro, Brazil) and Trolox® was supplied by Merck (Düsseldorf, Germany). All the reagents were of analytical grade and the stock solutions and buffers were prepared with Milli-Q purified water.

2.2. Honey sampling

For this study, fifteen honey samples, identified by numbers (**1**-**15**) from the Northeast region of Brazil, from Bahia state were analyzed. The samples of honey from *Apis mellifera mellifera* L. were collected by beekeepers from 7 counties (Table 1) within the micro-region Ribeira do Pombal-BA, during January to March, 2011 (period of increased production). All honey samples were processed by centrifugation method and kept at ambient temperature (26 ± 4 ^{o}C), until analysis within two weeks, protocol of storage followed by all beekeepers in the region.

Table 1. Distribution of the investigated honey samples (n = 15)

Localities	Coordinates		Number of samples	Identification of samples
	Latitude	Longitude		
Banzaê	10°35'S	38°37'W	01	**8**
Euclides da Cunha	10°30'S	39°00'W	03	**12,13** and **14**
Entre Rios	11°57'S	38°03'W	01	**15**
Heliópolis	10°41'S	38°17'W	01	**6**
Ribeira do Amparo	11°02'S	38°26'W	02	**2** and **3**
Ribeira do Pombal	10°50'S	38°32'W	04	**1, 4, 7** and **10**
Tucano	10°57'S	38°47'W	03	**5, 9** and **11**

Source.[43]

2.3. Sample floral-type identification

The preparation of honey samples was based on the pollen spectra proposed by Louveaux *et al*.[44] Ten grams of honey were dissolved in 20 mL of distilled water and centrifuged for 5 min at 3.000 rpm. The supernatant was discarded and 2 mL of glacial acetic acid were added in the test tube and left to stand for 24 h, and again centrifuged and the supernatant discarded.[45] The sequence analysis was proposed by Erdtman and Nilsson & Praglowski.[46-48] About 2 mL of the acetolysis mixture (9:1) [$(CH_3CO_2)O$:H_2SO_4)] were added in tubes that were placed in a water bath (100 °C, 5 min), and centrifuged (5 min, 3.000 rpm). After this step, 2 mL of distilled water and one or two drops of ethanol were added, then, the sediment was concentrated by repeated centrifugation. Approximately, 2 mL of glycerin – water (1:1) were added to the sediment, and then one more centrifugation was performed. The sediment was removed with the aid of a spatula, embedded in a glycerin jelly and deposited on a microscopic slide for subsequent quantitative and qualitative analyses. The examination of the slides was carried out with an optical microscope at 400× in order to identify the pollen types.

2.4. Physicochemical analysis

Samples were analyzed for moisture, total acidity, diastase activity, hydroxymethylfurfural (HMF) content, minerals, reducing sugars, apparent sucrose, free acidity, insoluble solids, pH, color and ash. All analyses were performed in triplicate.

The moisture content was determined based on the refractometric method.[46] All measurements were performed at 20 °C using an Abbe refractometer (digital refractometer Atago, Germany), after waiting for 6 min for attaining equilibrium, and obtaining the corresponding % moisture (g 100 g^{-1} honey) from the refractive index of the honey sample from a standard table (Chataway).[47]

Diastase activity was determined using a buffered solution of soluble starch and honey incubated in a thermostatic bath at 40 °C.[48] The diastase value was calculated using the time taken for the absorbance to reach 0.235, and the results were expressed in Gothe scale. Gothe's scale number is defined as g starch hydrolysed in 1 h at 40 °C per 100 g honey.[49,50,51,52]

Hydroxymethylfurfural (HMF) was determined by standard method.[46]

The ash content was determined by placing 5 g of honey samples in a crucible in a

muffle furnace and heating at 600 °C for 6 h, experiment performed in triplicate with the mean expressed in g%.[48]

Reducing sugars and apparent sucrose were determined by titration using the Fehling's test.[48,51]

The pH values of honey samples (CO_2 free water) were measured using a pHmeter (Basic 20) and the solution titrated with 0.05 mol L^{-1} sodium hydroxide solution up to pH 8.3, and free acidity was determined as usual.[46]

Insoluble solids were measured by gravimetric method.[47,48] The results were expressed in g 100 g^{-1} honey.

Honey color was determined by measuring the light transmittance of bubbles and debris free honey samples in a colorimeter (Hanna C221, Hungary) using glycerol as a standard. The results were expressed in mm Pfund.

2.5. Determination of total phenolic content

The total phenolic content (TPC) from honey samples was determined using Folin-Ciocalteau (FC) reagent, as described by Cicco *et al*,[53] with the following modifications. Aliquots (120 µL) of honey solutions (2.5 mg mL^{-1}) were placed in test tubes and 180 µL of water were added. Then, 300 µL of FC reagent were added to each tube. After 2 min, 2.4 mL of a 5 % (w/v) sodium carbonate solution were added. The mixture was shaken and heated at 40 °C in a water bath for 20 min. The tubes were then cooled rapidly and the developed color was read at 760 nm in a MultiSpec–1501 UV-Vis spectrophotometer (Shimadzu, Japan). The concentration of phenolic compounds was estimated using a calibration curve traced with gallic acid (GA) in water (4 - 40 µmol L^{-1}) as a polyphenol reference (n = 3). The results are expressed as mg of GA equivalents 100 g^{-1} of honey (mg GAE 100 g^{-1}). The same procedure was performed using 120 µL of water as a blank.

2.6. Radical scavenging activity of α,α-diphenyl-β-picrylhydrazyl radical (RSA-DPPH·)

The antioxidant capacity of honeys was measured as their radical scavenging ability (RSA), using the DPPH·method.[51,54] Thus, 0.30 mL of honey solution (2.5 mg mL^{-1}) was mixed with 2.7 mL of DPPH· radical solution (40 µg mL^{-1} in methanol) in a 3 mL quartz cuvette. The mixture was homogenized and stored in dark, prior to analysis. The DPPH· absorption values at 516 nm were recorded at 5 min intervals for 50 min. The percentage of DPPH radical-scavenging activity (RSA% - DPPH·) of each sample was calculated as follows: % RSA = $(1\text{-}A_C/A_D) \times 100$, where A_C is the absorbance of the solution when sample was added at a particular concentration in 30 min, and A_D is the absorbance of the DPPH·solution. All the determinations were performed in triplicate. The IC_{50} (half maximal inhibitory concentration) was calculated graphically, using a calibration curve in the linear range by plotting the sample concentration *versus* the corresponding scavenging effect (I%, inhibition percentage), for 30 min. The value of I% was calculated using the equation: $I\% = [(Abs_0 - Abs_1)/Abs_0] \times 100$, where Abs_0 is the absorbance of the control and Abs_1 the absorbance in the presence of the test compound.

2.7. FRAP assay

The assay was performed according to the method described by Rufino *et al.*[55] which is based on the reduction of a ferric tripyridyltriazine complex to its dark blue ferrous form, in the absence and presence of antioxidants. Briefly, the FRAP reagent is prepared by mixing 2.5 mL of a solution of 10 mmol L^{-1} TPTZ in 40 mmol L^{-1} HCl, and adding 2.5 mL of 20 mmol L^{-1} $FeCl_3$ and 25 mL of 0.30

mol L^{-1} acetate buffer (pH 3.6), after which, the reagent is heated to 37 ^{o}C. Aliquots (90 µL) of honey solutions (2.5 mg mL^{-1}) were mixed with 270 µL of distilled water and 2.7 mL of FRAP reagent and incubated at 37 ^{o}C for 30 min. The absorbance of the reaction mixture was measured at 595 nm and a calibration curve was prepared with Trolox® (0-30 µmol L^{-1}). The results are expressed as $TEAC_{FRAP}$, i.e., Trolox Equivalent Antioxidant Capacity, calculated with respect to the original FRAP, in µmol of Trolox 100 g^{-1} honey.

2.8. CUPRAC assay

These assays are based on the reduction of Cu(II) to Cu(I) by the combined action of all the antioxidants (reducing agents) in a sample. 1 mL each of $CuCl_2$ (1×10^{-2} mol L^{-1}), neocuproine (7.5×10^{-3} mol L^{-1}) and NH_4Ac buffer solutions (1.0 mol L^{-1}, pH 7.0) were placed in a test tube. Then, 0.5 mL of the honey solution (or Trolox®, for calibration curve) and 0.6 mL of H_2O were added to the initial mixture to reach a final volume of 4.1 mL. The tubes were stoppered, and after 1 h, the absorbance at 450 nm was recorded against a blank reagent. The calibration curve was prepared with Trolox® (0-73 µmol L^{-1}).[53,56] The results are expressed as $TEAC_{CUPRAC}$, in µmol of Trolox® 100 g^{-1} of honey.

3. Statistical Analysis

All the analyses were carried out in triplicate and the results were reported as mean ± standard deviation. Analysis of variance and least significant difference tests were conducted to identify differences among means, while a Pearson correlation test was conducted to determine the correlations among means; p-value <0.05 was regarded as significant. Statistical analysis was performed using SAEG.[57]

4. Results and Discussion

4.1. Sample floral-type identification

The palynological analysis is very important for the differentiation of honey produced in different geographical and climatic areas. Table 2 lists the percentage of pollen from different plant species in the samples of honey. The characteristics of the melissopalynological analyses confirmed the floral origin of the samples. Among the 15 samples, thirteen were classified as polifloral consisting of a mixture of pollen from different plant species, while samples **8** (*Pityrocarpa moniliformes* (Fabaceae)) and **15** (*Eucalyptus* sp. (Mirtaceae)) were monofloral. Monofloral status refers to the presence of a single pollen type in quantities > 45% of the total pollen in the spectrum.[30] However, this is not the only parameter. In our work, the monofloral honey was confirmed by observing the high frequency (> 45%) of pollen types associated with the identification of the pollen type of plant species.

The samples were produced in the semi-arid region of Bahia state which has a great bee potential due to the diversity of flora. The *Myrcia* pollen type was found in 93.33% of the investigated samples and the *Eucalyptus* pollen type was only present in sample **15**. Our results indicated that the distribution of the pollens greatly varies among honey samples confirming the influence of the diversity of the flora of the semi-arid region. According to Barth,[58] due to the great extension of a country, there is a great variety of honeys, so it is not possible to come to general conclusions. This matter is always of regional concern, with the tendency to address “micro” regions of honey production.

Table 2. Characterization of the analyzed melissopalynological honey samples

Samples	Type of honey	Pollen type	Percentage (%) and classes'frequency	Quantity of pollen types
01	Polifloral	*Mimosa tenuiflora*	(32.50 AP**)	20
02	Polifloral	*Myrcia* 1	(54.38 DP*)	20
03	Polifloral	*Myrcia* 1	(55.00 DP)	9
04	Polifloral	*Hyptis multiflora*	(23.7 AP)	20
05	Polifloral	*Myrcia* 1, *Vernonanthura*	(23.01AP), (17.57 AP)	19
06	Polifloral	*Heliotropium, Salvia*	(25.18 AP), (24.71 AP)	8
07	Polifloral	*Myrcia* 1	(79.57 DP)	4
08	Monofloral	*Pityrocarpa moniliformes* (quipé), *Piptadenia stipulacea*	(74.15 DP), (18.25 AP)	7
09	Polifloral	*Myrcia* 1, *Mimosa tenuiflora*	(25.20 AP), (20.22 AP)	25
10	Polifloral	*Myrcia* 1	(75.21 DP)	5
11	Polifloral	*Myrcia* 1, *Mimosa tenuiflora*	(25.98 AP), (19.30 AP)	18
12	Polifloral	*Myrcia* 1, *Gochnatia*	(49.63 AP), (37.99 AP)	13
13	Polifloral	*Gochnatia*, *Myrcia* 1	(52.17 DP), (42.93 AP)	7
14	Polifloral	*Myrcia* 1, *Gochnatia*	(56.46 DP), (30.93 AP)	13
15	Monofloral	*Eucalyptus*, *Myrcia* 3	(68.47 DP), (26.63 AP)	3

*DP, dominant pollen (> 45% of total grains)

**AP, accessory pollen (16-45%)

4.2. Physicochemical analysis

Table 3 lists the results obtained for the physicochemical parameters analyzed in the 15 samples of honey. Data show that the studied honey samples are of good quality. The moisture content is one of the main parameters of quality analysis of honey. Moisture is the second component in an amount which varies depending on the climate, floral origin and harvest time.[50] The moisture content (%) in the investigated samples ranged from 17.46 to 20.28, which is within the imposed limit ≤ 20%.[49,50] Higher contents can lead to honey fermentation during storage. The results are similar to other studies.[30,59,60] Ash content is within the maximum limit of 0.6%. Concerning the total reducing sugars, all samples varied from 74.54 to 83.93%. These values were similar to the 70.38 to 87.39% obtained in honey samples from Piauí, [61,62] north of Brazil. As such, all samples were above the 65% lowest limit allowed in Brazil.[50] Apparent sucrose (maximum limit of 6%), insoluble solids (maximum limit of 0.1 g 100 g^{-1}), free acidity (maximum limit of 50 mEq kg^{-1}) values were 12.77 to 55.72 (mEq kg^{-1}), similar to the ones (30.4 to 58.4) reported in samples of Spanish honey.[63] Despite not being required by Brazilian legislation,[50] for pH (range of 3.20 to 4.60), the values were within the allowed Brazilian standards, like in honey from cashew flowers, around, 3.67.[40]

The content of HMF in honeys is indicative of freshness of honey samples.[30] Values of HMF ranged from 2.40 to 37.87 mg kg^{-1}, while the allowed limit is 60 mg kg^{-1}.[50] These results were similar to other studies, for instance, for cashew flowers, from Ceará State,[40] with values around 14, for Algerian honeys,[64] which values vary from 15.23 up to 24.21 mg kg^{-1}. It is well known that heating of honey results in the formation of HMF, which is produced during acid-catalyzed dehydration of hexoses, e.g. fructose and glucose.[65] In Malaysian honey samples,[4] HMF concentrations in fresh samples (stored within six months around 25-30 ^{o}C) varied from 2.80 to 24.87 mg kg^{-1}, i.e., within the

internationally recommended range (80 mg kg^{-1} for tropical honeys). However, the same honey samples, when stored from 12 to 24 months, were shown to have much higher HMF concentrations, ranging from 118.47 up to 1139.95 mg kg^{-1}, which indicate that the shelf validity (at room temperature) is limited.[66]

Table 3. Physicochemical parameters of honey

Honey sample	HMF (mg kg^{-1})	Moisture content (%)	Minerals (%)	Reducing sugars (%)	Apparent sucrose (%)	Diastase activity (Gothe)	Free acidity (mEq kg^{-1})	Insoluble solids (g 100 g^{-1})	pH	Color (mm pfund)
1	37.87	18.50	0.248	75.66	1.41	21.66	45.4	0.019	3.72	91
2	10.55	20.28	0.27	75.08	0.51	24.28	40.22	0.025	4.17	150
3	15.49	19.07	0.237	79.44	1.92	12.09	40.56	0.067	4.10	135
4	19.23	18.61	0.274	77.52	1.06	19.98	45.22	0.025	3.90	91
5	6.89	18.86	0.472	74.54	0.98	25.58	34.06	0.022	4.14	102
6	29.18	17.85	0.19	75.49	1.34	23.66	37.67	0.016	3.89	74
7	16.91	19.14	0.264	75.59	2.63	22.76	38.01	0.019	3.75	87
8	4.34	17.93	0.075	76.77	2.55	8.83	12.77	0.014	3.8	12
9	27.47	18.36	0.219	78.32	1.70	4.52	38.19	0.023	3.78	81
10	23.73	18.89	0.188	77.26	2.42	14.44	36.5	0.018	3.83	96
11	12.95	18.67	0.225	78.89	2.9	22.12	40.46	0.003	3.98	116
12	27.09	18.24	0.305	78.98	2.49	0.13	48.04	0.007	3.72	72
13	2.99	18.55	0.309	79.81	1.5	0.14	55.72	0.011	3.65	73
14	32.33	17.46	0.246	83.93	1.79	ND	47.99	0.010	3.69	72
15	2.40	17.47	0.293	79.60	1.76	11.57	14.57	0.011	4.25	34

The diastase activity is a parameter related to the maturity and freshness of honey and diastase (α-amylase) is an enzyme present in honey.[67] Honey samples exhibited very different values, ranging from 0.13 up to 25.58 in Gothe scale. Only four samples (**9, 12, 13** and **14**) presented an inappropriate diastase activity (< 8 in Gothe scale). Ozcan,[17] suggested that the enzyme activity below this limit can be explained by a variety of reasons, including the amount of sucrose in food source, rate of nectar flow and even age of the bees. Different values were found in several regions. In Turkey honeys,[68] variations from 3 to 70.4 were found, while in Silva et al.,[34] in honey samples from Portugal, the values ranged from 3 to 38, in Gothe scale.

In Brazil, the diastase activity should be at least 8 on a Gothe scale. Honeys, with low enzyme content, should have at least a diastase activity corresponding to three ranges of Gothe, while HMF content does not exceed 15 mg kg^{-1}.[50]

The color of honey is one of the main sensory characteristics, with great attractiveness for consumers. It depends on various factors, such as their mineral content,[69] pigment content (carotenoids and flavonoids),[70] botanical origin, suspended particles as pollen.[71] It can also be affected by heat, as an increase in temperature catalyses melanoidin formation and caramelisation reactions, time of storage, and antioxidant activity.[20] Phenolic compounds can greatly contribute to the colour, especially when complexed to metals, notably iron.[20] The results of color analyzes of the honey samples revealed a percentage of 33% for each amber and light amber and 20% for dark amber, while for extra white and white, 7% of

each was found. Thus, there is a predominance of amber color in the present case. Lacerda,[72] analyzed 24 samples of honey from Vitoria da Conquista-BA and found a predominance of white (29%) and dark amber (25%). It is interesting to note that samples **8** and **15** are the lightest (Table 3) and they were classified as monofloral. In Sant'Ana *et al*., [20] colour was also classified according to visual analysis and Pfund scale. They analyzed 60 Brazilian *Apis mellifera* honeys from different floral origins and obtained from different parts of the state of Rio de Janeiro. Most of the honeys were considered to be amber or dark amber by the Pfund classification. González-Miret *et al*.[73] showed that light coloured honeys generally have less than 2.79 mg/kg of iron, while amber honeys have an average of 2.84 mg/kg and dark coloured honeys have more than 4.26 mg/kg.

4.3. Determination of total phenolic content (TPC) and antioxidant capacity

Several studies have linked the presence and amount of polyphenols with nutrition and human health. [3,7-12,19,28-39] Table 4 shows TPC, DPPH, FRAP and CUPRAC results obtained from 15 honey samples. The TPC values ranged from 27.0 to 92.7 mg GAE $100g^{-1}$ of honey, being the highest values found for samples **2**, **5** and **11** (all classified as polifloral honeys), when compared to samples **8** and **15** (monofloral honeys). Variations in TPC may be due to different floral origins. These results were similar to other studies reported in literature (Table 5).

All samples have radical scavenging ability (Table 4 and Fig. 1), with a consumption of DPPH ranging from 7.3% to 25.9%, in 30 min, compared to standard gallic acid, consuming 100% of the radical.

It should be emphasized that the comparison on antioxidant capacity among honeys is, in large extension, difficult, due to the use of different protocols, standards and unities used. Systematization is urgently required. The values diverge too much (Table 5), depending on several factors, as mentioned before. Al et al.,[74] for Romanian honeys, found RSA values from 35.8% to 49.1% while, Baltrusaityte *et al.*,[28] for Lithuanian honeys, obtained variations from 36.5% to 86.90%. In our work, IC_{50} values of honey samples varied from 8.2 to 14.5 µg mL^{-1} (Table 4), comparable to those reported in the literature (Table 5), mainly from tropical regions, like the ones from Roraima and Malaysia.

The reducing capacity of the samples was evaluated by FRAP and CUPRAC methods (Table 4, Fig. 1). The FRAP values varied from 99.4 to 720.4 µmol TEAC 100 g^{-1} honey and for CUPRAC between 338.7 to 960.0 µmol TEAC 100 g^{-1}. The lowest reducing capacity was obtained for sample **8** for both methods. The highest FRAP value was obtained for samples **13** followed by **14** and **12**. For CUPRAC, the highest capacity was obtained for samples **2-3** and **13-14** (Fig. 1). All samples have reducing capacity, with emphasis on samples collected in the municipalities of Ribeira do Amparo (samples **2** and **3**) and Euclides da Cunha (**12, 13** and **14**), where there is a predominant semi-arid vegetation.

Table 4. TPC, DPPH, FRAP and CUPRAC of honey samples

Honey Sample	TPC	DPPH•		FRAP	CUPRAC
	mg GAE*100 g $^{-1}$ of honey	RSA% **	IC_{50} (mg mL^{-1})	TEAC***$_{FRAP}$ (µmol TE 100 g^{-1})	TEAC***$_{CUPRAC}$ (µmol TE 100 g^{-1})
1	41.1 ± 3.5 h	16.7 ± 0.7 e	-	241.6 ± 4.8 h	556.3 ± 22.2 f
2	81.5 ± 2.1 c	20.5 ± 0.9 d	8.2±1.3	563.7 ± 37.0 c	960.1 ± 11.0 a
3	76.0 ± 0.9 d	23.3 ± 0.8 b	12.1±0.2	513.3 ± 23.1d	926.2 ± 9.7 a
4	74.0 ± 3.6 d	7.3 ± 1.2 i	-	302.4 ± 3.4 g	619.8 ± 15.9 e
5	83.0 ± 1.3 b	15.0 ± 0.9 g	14.4±0.1	325.5 ± 7.2 f	595.1 ± 6.2 f
6	43.0 ± 4.4 g	17.0 ± 0.3 e	-	165.1 ± 17.3 i	462.1 ± 17.4 g
7	40.0 ± 2.1 h	15.6 ± 0.0 f	-	195.2 ± 8.5 i	453.0 ± 3.8 g
8	27.0 ± 2.3 i	13.1 ± 0.7 h	-	99.4 ± 3.8 j	338.7 ± 8.45 h
9	51.0 ± 0.8 f	20.6 ± 0.6 d	12.9±0.04	518.0 ± 10.7 d	628. 5± 11.5 e
10	57.0 ± 1.9 e	18.9 ± 0.1 d	14.5±0.03	392.1 ± 8.6 e	578. 1± 32.4 f
11	92.7 ± 2.1 a	21.4 ± 1.7 c	14.4±0.06	347.9 ± 8.4 f	672. 9± 17.1d
12	51.0 ± 1.4 f	20.1 ± 0.8 d	12.0±0.05	597.6 ± 19.9 b	730.6 ± 42.5 c
13	46.0 ± 3.4 g	25.9 ± 0.2 a	13.7±0.07	720.4 ± 23.8 a	793. 2± 13.7 b
14	51.0 ± 0.4 f	22.4 ± 0.9 c	12.5±0.07	603.6 ± 15.4 b	796.9 ± 46.8 b
15	31.0 ± 1.3 i	17.1 ± 0.9 e	-	181.4 ± 21.6 i	592.8 ± 17.2 f

*Gallic Acid Equivalents. ** Percentage of DPPH radical-scavenging ability in 30 min. ***TEAC: Trolox Equivalent Antioxidant Capacity (µmol TEAC 100 g^{-1} honey). [1]Values are mean ± SD. Means with different letters within a column are significantly different (p<0.05).

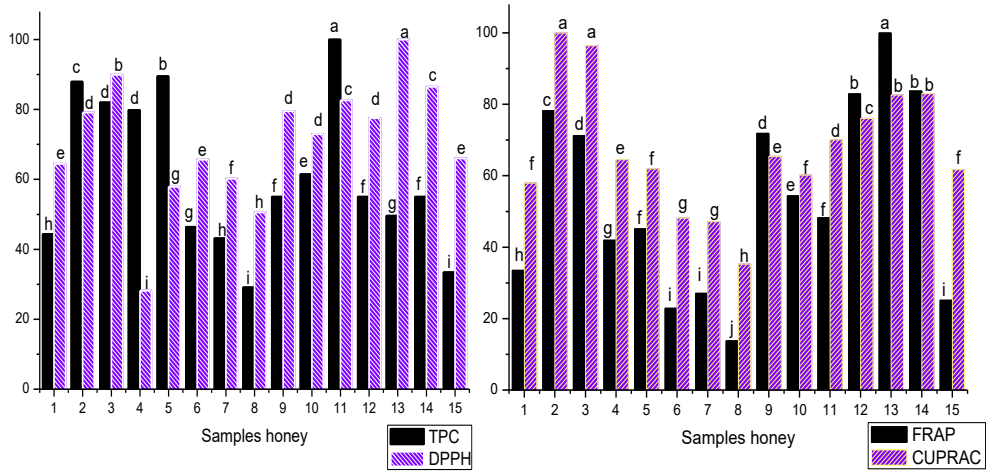

Figure 1. Normalized parameters of the honey samples, in relation to each antioxidant test. The number of the sample is located between the values of TPC and DPPH or between TRAP and CUPRAC. The highest activity was considered 100% and the other values correspond to a relative percent of activity. Within each column, averages denoted with the same letter were not significantly different by this test (p< 0.05)

Table 5. Comparative results of TPC and DPPH among honeys from different parts of the world

Localities	TPC (mg *EAG/ 100 g of honey) (variation)	DPPH IC_{50} (mg mL^{-1}) or % inhibition (variation)	References
Brazil	27.02 ± 2.3 to 92.70 ± 2.1	8.2 ± 1.3 to 14.5 ± 0.03 (7.3% to 25.9%)	Present work
Romenia	2.00 - 125	40.67% – 64.83%	74
Brazil	34.0 ± 1.8 to 78.2 ± 2.7	10.81 ± 0.50 to 52.64 ± 4.70	75
Brazil	61.11 ± 0.07 to 141.06 ± 0.31	10.19 ± 1.65 to 83.01 ± 1.46	76
Brazil (Rio de Janeiro, SE Brazil)	95.88 ± 16.92 to 115.14 ± 20.13	16.95 ± 7.28 to 25.02 ± 9.37	20
Brazil (Roraima)	25.0 ± 3.24 to 54.8 ± 3.00	3.17 to 8.79	77
Italy	17.1 to 60.0	5.0 to 15.5	78
Italy	11.08 ± 2.80 to 14.26 ± 4.14	55.06 ± 7.04% to 75.37 ± 7.87%.	79
Italy	6.6 ± 0.7 to 38.9 ± 7.9	7.08 to 64.09	80
Italy	15.13 ± 3.93 to 82.49 ± 4.05	11.39 ± 2.11 to 64.33 ± 2.76	81
Italy	6.05 ± 3.63 to 27.60 ± 13.80	7.08 ± 0.35 to 64.09 ± 2.56	82
Africa	5.25 ± 1.5 to 78.96 ± 13.8	1.63 ± 0.17 to 47.62 ± 0.39	83
Africa	32.6 ± 0.48 to 114.8 ± 1.30	1.37 ± 0.03 to 29.13 ± 1.5	84
Slovenia	4.48 ± 14.8 to 24.14 ± 39.5	7.2 ± 1.2 to 53.8 ± 8.5	85
Malaysia	1.52 ± 0.51 to 5.26 ± 1.21	4.71 ± 0.36 to 18.58 ± 0.80	4
Malaysia (Tualang)	27.75 to 83.96	5.80 to 10.86	86
Poland	44.3 ± 5.17 to 177 ± 79.4	47.2 ± 8.75 to 83.4 ± 4.11	87
Poland	14.28 ± 16.0 to 111.3 ± 52.0	_	88
Argentina	18.73 to 107.32	_	89
Portugal	13.22 ± 0.05 to 72.78 ± 0.23	84.98 ± 1.19 to 168.94 ± 19.20	90
Portugal	31.85 ± 2.21 to 117.65 ± 2.21	_	91
Pakistan	36.01 ± 0.22 to 252.00 ± 1.61	30.52 ± 0.31 to 77.43 ± 0.77	92
Serbia	30 to 139	(1.31% to 25.61%)	93
Serbia	9.4 ± 3.1 to 62.07 ± 0.8	(0.22 ± 0.01 to 1.21 ± 0.02 μmol TE/g)	94
Venezuela	38.15-182.10	_	95
India	47± 0.2 to 98 ± 1.2	(44% to 71%)	96
Czech Republic	8.63 ± 0.20 to 24.25 ± 0.06	9.87 to 44.20 mg EAscorbic Acid 100 g^{-1})	97

The samples were grouped according to the following dendrogram (Fig. 2). It is noted that the 15 samples were divided into five groups: one group with 2 samples (**2** and **3**), group 2 with 4 samples (**9**, **12**, **13** and **14**), group 3 with 3 samples (**1, 6,** and **7**), group 4 with 2 samples (**8** and **15**) and the fourth group with 4 samples (**4**, **5**, **11** and **10**). The proximity of the samples suggests similar physical and chemical characteristics, such as those present in the samples within each group formed. Moreover, the distances between the samples show physicochemical differences between them, such as those between groups.

Cluster analysis has been used by Moreti,[98] to study honey samples of the State of Ceará, and Sodré,[61] in honey samples of Piauí State, helping to verify that the samples of the same floral origin were grouped in the same groups or subgroups.

In this work, it appears that in group 1, samples from the same source, county of Riverside Support samples, were grouped. Samples **2** and **3** have, in common, darker colors, in pH values above 4.1, acidity greater than 40 mEq kg^{-1}, high content of total phenols, high antioxidant capacity, statistically superior antioxidant capacity through analysis by FRAP method. It is observed that the samples, classified as monofloral, were grouped into the same group 4 with two samples (**8** and **15**). They have in common, a lighter color and lower antioxidant activity. In Group 5, the samples from the same locality, the municipality of Euclides da Cunha, which has a savanna vegetation, have in common, high antioxidant activity, low diastatic activity and are polifloral.

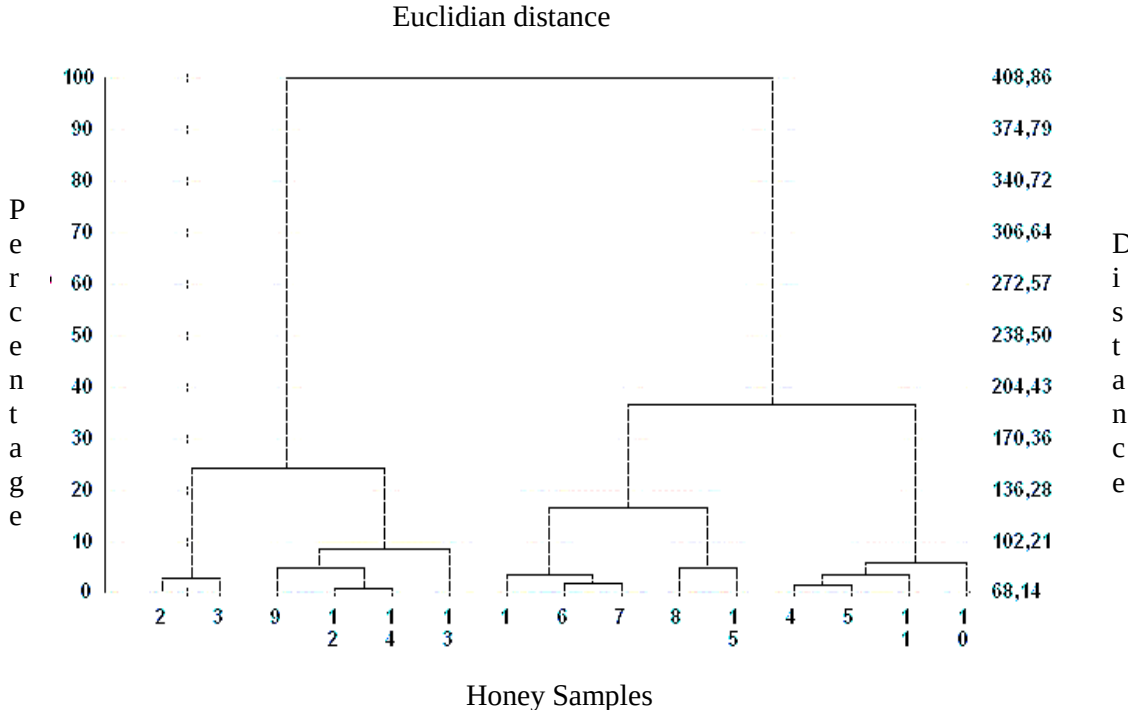

Figure 2. Dendrogram of the cluster analysis using the average Euclidean distance and UPGMA for 15 samples of honey produced by *Apis mellifera* in the northeastern state of Bahia, in relation to physicochemical characteristics. Group 1 has two samples (**2** and **3**); group 2, 4 samples (**9,12,14** and **13**); group 3, 3 samples (**1,6,7**); group 4, 2 samples (**8** and **15**) and finally group 6, with 4 samples (**4,5,11** and **10**)

5. Statistical Results

The analysis of variance and least significant difference tests showed statistically significant differences among the samples for the different methods (Table 4 and Fig. 1). These results had shown that sample **11** was the best for TPC, the sample **13,** in DPPH and FRAP assays and samples **2** and **3,** for CUPRAC. The correlation among TPC, DPPH, FRAP and CUPRAC and between these with color in honey samples were established. A statistically significant correlation (p<0.05) was observed between TPC with CUPRAC (0.55), DPPH with FRAP (0.73) and CUPRAC (0.64), FRAP with CUPRAC (0.83). This suggests that all assays are recommendable for evaluating antioxidant capacity in honey samples. Also, the correlations between the color with TPC, DPPH, FRAP and CUPRAC were performed. The color showed significant correlation (p<0.01) with TPC (0.82) and CUPRAC (0.66). Interestingly, the lighter honey samples (**8** and **15**) had the lowest TPC values, while samples classified as dark amber (**2**, **3** and **11**) presented the highest TPC, suggesting the influence of polyphenols on honey color. The existence of positive correlations between the color of honeys and their antioxidant properties has been demonstrated.[6,20,29,39,99,100]

In Santana *et al*.,[20] linear relationships were observed between colour and flavonoid content, total phenolics and antioxidant capacity, and total flavonoid and phenolic contents. The white-coloured *Citrus* honey showed the lowest antioxidant activity, while the light ambar Verbenaceae honey showed the highest total phenolics and antioxidant activity. Dark-coloured and polyfloral honeys, though less popularized among consumers, showed average to high antioxidant capacity.[20] In Polish honeys,[88] the honeys' color ranged from pale yellow to dark amber. Dark buckwheat honey with much higher phenolic content (111.30 mg GAE/100 g) exhibited higher antioxidant activity.

The correlation obtained between the antioxidant capacity and the total polyphenolic content suggests that the phenolic compounds are, in great part, responsible for the antioxidant effects of honey[99,100] These results suggest that honey may be used as a healthy alternative to sugar in many products and thereby serves as a source of dietary antioxidants. Honey with high levels of antioxidants can provide protection to healthy human subjects.[101]

6. Conclusions

The present study showed that honey samples from the micro-region of Ribeira do Pombal (Bahia state, Brazil) have good antioxidant potential. Among the 15 honey samples, 13 were classified as polifloral consisting of a mixture of pollens from different plant species, while samples **8** (*Pityrocarpa moniliformes* (Fabaceae)) and **15** (*Eucalyptus sp.* (Mirtaceae)) were monofloral honeys. The *Myrcia* pollen type was found in 93.33% of the investigated samples and the *Eucalyptus* pollen type was only present in sample **15**. Our results indicated that the distribution of the pollens greatly varies among honey samples confirming the influence of the diversity of the flora of the semi-arid region. Physicochemical parameters are within the allowed standards established by Brazilian law. Only four samples (**9**, **12**, **13** and **14**) presented an inappropriate diastase activity. There is a predominance of amber color for samples. Honey samples with dark amber and amber colors present higher antioxidant capacity than light color honeys. Also, these honeys were classified as polifloral. All samples presented good total phenolic content and antioxidant capacities, being the highest performance obtained for the darker and polifloral ones. Positive correlations were found among total phenolic content, antioxidant capacities and color. The increase of the color intensity seems to be related to increase on the concentration of

polyphenols.. The importance of honey has been continuously upgrading because of its nutrient and therapeutic effect, which, in turn is directly related on its phenolic content and antioxidant capacity. These properties should be publicized to increase comsumption among the population. It is important to emphasize that the honey produced in the microregion Ribeira do Pombal contributes to the development of regional economy and provide an alternative to family income increase.

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